

# Plasticizers in Blood—Real or Artifactual?

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During our recent work on the identification of drugs involved in overdose cases (1, 2), we have had occasion to investigate the constituents found in blood, urine, and gastric juices by combined GC-mass spectrometry. In a surprisingly large number of cases, we have observed, in addition to any drugs present, one or more peaks corresponding to phthalate esters (PAEs). Most commonly these were di-*n*-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP), but occasionally di-2-ethylhexyl adipate and butyl cellosolve phosphate were also present. Often they were present at remarkably high levels, e.g., 200 mg/100 ml in blood. In view of the many possible sources of these plasticizers in the hospital and laboratory environment, we deemed it advisable to study in more detail blood samples from normal individuals, taking special care to prevent the introduction of such compounds from outside sources during sample workup.

## Experimental

Samples of blood were drawn by two methods. In the first case, bare syringe needles were inserted into the blood vessel and the blood allowed to drip directly into a

carefully cleaned and chloroform-rinsed glass tube. In the other method, samples were drawn by an all-glass syringe with the use of a metal needle. Plastic Vacutainer assemblies (Beckton-Dickinson Co., Rutherford, N. J.) were not used for this purpose, since the small plastic needle sleeve of the assembly was found to contain diethyl phthalate. In several cases, anticoagulants, such as heparin or EDTA, were added to prevent clotting. Other samples were not so treated. In all cases 10 ml samples of blood were shaken with 8 ml of chloroform which previously had been concentrated and analyzed by GC-MS and found to be completely free of plasticizers. The mixture was then centrifuged to separate the phases and the chloroform layer removed with a chloroform-rinsed disposable pipet, after which the chloroform was partially evaporated at room temperature under a stream of nitrogen. The solution was reduced to approximately 50  $\mu$ l, and a 2- $\mu$ l aliquot was injected directly onto a 1% OV-17 column on a Glowall gas chromatograph which had been calibrated by using DEHP and dibutyl phthalate. Under the conditions of the analysis, 0.1  $\mu$ g of DEHP, corresponding to a blood level of 25  $\mu$ g/100 ml, would have given a peak approximately 5 cm high. In every case a large peak for cholesterol was observed, eluting later than that of the DEHP, and it was necessary to let this peak emerge between samples to avoid confusion. In many cases additional peaks were observed, and when it was considered possible that these might be

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other types of plasticizers, the sample was injected into the LKB GC-mass spectrometer. In no case did the resultant mass spectrum indicate the presence of any plasticizers.

## Discussion

From the dilutions involved, it is our feeling that we would have been able to easily observe a level of 0.025 mg-% in blood. Twenty-five individuals, randomly selected, were investigated in this manner, and in no case was any evidence obtained for the existence in their blood of the common plasticizers.

We therefore can conclude that the level of plasticizers in blood must be below the aforementioned value, and they are, therefore, not expected to be found at levels comparable to therapeutic levels of most modern drugs.

Several laboratory sources of contamination were finally identified. In one case, filter papers used to facilitate phase separation between chloroform and water were found to contain phthalate esters at rather

high levels. In another case, phthalate esters were found in bulk quantities of alcohol used in early chloroform-ethanol extractions. In a third case, di-2-ethylhexyl adipate was observed to be present in the plastic wrappings at the top of solvent bottles.

While there seems to be no doubt whatsoever that plasticizers are to be found in blood samples where the blood has been stored or in contact with plastic apparatus, such as blood bags, etc., we conclude on the basis of our experiments that indications of plasticizer in humans under normal conditions should be viewed with a great degree of caution.

## REFERENCES

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